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(54) Title: SYNTHETIC FIBRONECTIN FRAGMENTS AS INHIBITORS OF RETROVIRAL INFECTIONS			
(57) Abstract			
<p>The invention includes methods for inhibiting retroviral infections such as HIV. The methods of this invention involve the use of certain fragments of fibronectin and such fragments conjugated to carrier molecules such as ovalbumin to inhibit retroviral infections. The invention also includes novel proteins which comprise fibronectin fragments covalently linked to carrier proteins.</p>			

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SYNTHETIC FIBRONECTIN FRAGMENTS AS INHIBITORS OF RETROVIRAL INFECTIONS

BACKGROUND OF THE INVENTION

5

Field of the Invention

This invention is in the field of chemical agents and methods of inhibiting infection of cells by retroviruses.

10 Description of the Prior Art

Integrins represent a family of cell surface $\alpha\beta$ heterodimeric proteins that mediate cell adhesion to other cells and to extracellular matrix constituents including fibronectin (FN). Integrins composed of $\beta 1$ and $\beta 3$ subunits complexed to $\alpha 3$, α , and $\alpha 5$ subunits interact with the arginyl-glycyl-aspartic acid (RGD) sequence of FN7 whereas $\alpha 4\beta 1$ is specific for RGD-independent sequences in the carboxyl terminal cell and heparin-binding domain of fibronectin that mediates cell attachment. However, recent evidence suggests that cellular recognition and adhesion to fibronectin domains is more complex, involving not only integrins, but also cell surface proteoglycans (PG). PG are complex macromolecules consisting of a core protein to which highly anionic glycosaminoglycan (GAG) chains are covalently attached by O-linkage to serine or threonine residues. The anionic properties of GAG on many cell surface PG are important for binding to their respective ligands. Clearly, cell interactions with FN may involve several distinct sites, and this has been shown by the use of synthetic FN peptides. Cell binding to an A-chain-derived 33 kD carboxyl terminal heparin binding fragment of human plasma fibronectin of 66 KD B chain may involve both cell surface $\alpha 4\beta 1$ integrin and PG since this FN fragment contains cationic peptides, which bind cell surface chondroitin and heparin proteoglycans (CSPG and HSPG respectively) and/or alpha and beta integrins directly. Figure 1 shows a schematic diagram depicting the location of the 33 kD carboxyl terminal heparin binding fragment within fibronectin and the synthetic peptides described in this application. In contrast to peptides FN 1-5 and FN7, which are present within type III homologies common to all isoforms of fibronectin, peptide FN6 is restricted to isoforms of fibronectin which contain type IIIcs region (A-chains of human

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plasma fibronectin and human cellular or "tissue" fibronectin). The amino terminal end and the carboxyl terminal limit of the 33 kD fragment is based on known sequence data. Selected biological domains, indicated in Fig. 1 by roman numerals at the top of the figure, are based on the nomenclature of Furcht, 1981. I=weak heparin binding, II=collagen binding (noncovalent), III=free sulfhydryl, IV=RGD-mediated cell adhesion, V=carboxyl terminal strong heparin binding and cell adhesion, VI=free sulfhydryl. Approximate locations of tryptic (T) and cathepsin D (C) sites on intact fibronectin are shown. The $\alpha 4\beta 1$ integrin also binds to VCAM, which is an Ig superfamily receptor expressed on certain cells, including activated endothelial cells. Sequences of fibronectin peptides may interfere with alpha 4 beta 1 VCAM dependent interactions or other integrin, selectin or CAM dependent interactions.

Human immunodeficiency virus type 1 (HIV-1), a retrovirus, is the etiologic cause of AIDS. The HIV-1 envelope glycoprotein, gp120, binds to the CD4 receptor on T lymphocytes and on mononuclear phagocytes leading to infection of these target populations. Although infection of T lymphocytes requires cellular proliferation and DNA synthesis, productive infection of monocytes can occur independently of cellular DNA synthesis. Moreover, infection of activated CD4+ lymphocytes causes cell death, whereas infected monocytes are relatively resistant to destruction by the virus, serving as long-lived reservoirs of HIV. These cells not only provide a source of replicating virus, but their dysfunction following infection may contribute to increased susceptibility to opportunistic infections that are the hallmark of AIDS. Because monocyte-macrophages serve as reservoirs for HIV-1, selective targeting of this population, in addition to T lymphocytes, needs to be considered.

Zidovudine (AZT), an analogue of thymidine, dideoxyinosine (ddI), and dideoxycytosine, dideoxycytosine (ddC) are the primary anti-viral drugs currently in use for the treatment of HIV infection. These agents inhibit viral replication, but are unable to eliminate the virus. In addition, bone marrow toxicity, loss of efficacy with time and emergence of resistant HIV strains with available therapies have necessitated continued exploration for alternative drugs for the treatment of HIV infection. We have recently identified a novel mechanism for inhibiting HIV

-3-

infection utilizing fibronectin and/or its fragments which provide the basis for therapeutic intervention. There is an extensive body of literature related to AIDS research.

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Su et al., *Mol. Immunology*, Aug. 1991, 28(8):811-817 describe the binding of fibronectin to gp 120.

15

SUMMARY OF THE INVENTION

This invention describes the use of fibronectin fragments and conjugates with carrier molecules thereof for preventing or treating retroviral infections and associated diseases including AIDS. Selected peptides derived from fibronectin are effective inhibitors of HIV infection, replication and/or transmission. Addition of fibronectin peptides to cultures of human peripheral blood mononuclear leukocytes during exposure to HIV results in inhibition of infection as measured by reverse transcriptase activity in the cultures when compared to untreated infected cultures. This activity demonstrates the ability of the peptides to block viral adherence to and infection of mononuclear leukocytes.

20

More specifically, the invention encompasses carrier molecules covalently linked to an amino acid sequence selected from the group consisting of FN 1, 2, 3, 4, 5, 6 and 7 as herein after defined. The invention also encompasses pharmaceutical compositions for treating retroviral infection. The invention also encompasses methods for inhibiting retrovirus infection of mammalian cells by contacting the cells with an effective inhibiting amount of FN 1, 2, 3, 4, 5, 6 or 7 or these peptides

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conjugated to a carrier molecule. Furthermore, this invention encompasses the use of the compounds with conventional AIDS therapy such as AZT, ddi, and ddc. Those skilled in this art will recognize that peptides homologous to the above fragments, their derivatives and their conjugates with carrier molecules will have equivalent activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the location of synthetic peptides within intact fibronectin.
Figure 2 shows the inhibition of HIV infection by fibronectin peptides and anti CD4.
Figure 3 shows fibronectin peptides inhibiting HIV infection.
Figure 4 shows how xyloside augments anti CD4 inhibition of HIV infection.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods for preventing retrovirus infection, particularly HIV infection of mammalian cells, and associated diseases. Two of these synthetic peptides (FN-1 and FN-2) which are also in the B chain of fibronectin bind heparin and promote cell adhesion, implicating cell surface PG or other carbohydrate binding molecules in mediating cellular recognition of these two peptides. Additionally, a third synthetic peptide, FN-6, is located in close proximity to FN-1 and FN-2 and it promotes cell adhesion by an $\alpha 4\beta 1$ integrin-dependent mechanism.

Additional peptides from within the 33 kD fragment of the A-chain and are also seen in the B-chain (FN-3, FN-4, FN-5) also influence cell adhesion properties. In addition to influencing cell-virus interactions and cell to cell interactions, functional adhesion molecules and cell-matrix interactions have been shown to be vital to localization of immune and infected cells to target tissues. This increased adhesion to the basement membrane proteins, laminin and Type IV collagen, as well as fibronectin, implicate these pathways in coordinating the early margination, recruitment and migration of leukocytes to tissue sites with the potential for viral spread. Consequently, methods to interfere with these pathways and their

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contribution to the immune deficiency syndrome have a beneficial effect on this devastating disease.

Mononuclear leukocytes (10^7) were co-cultured with macrophage tropic HIV_{Ba-L} in adherent monolayers. The cells were then washed and re-fed in DMEM (Gibco Laboratories, Grand Island, NY) with 50 μ g/ml gentamicin, 2 mM glutamine, containing 10% FCS. Media (50%) were removed and fresh medium added at 3-4 day intervals. Infection was monitored measuring by reverse transcriptase (RT) at 3- to 4-day intervals.

Peptides FN-1, 2, 3, 4, 5, 6, 7 were coupled to ovalbumin (OA) as a biological carrier molecule using 1-Ethyl-3-[3-Dimethylaminopropyl]-carbodiimide, hydrochloride, dialyzed, and diluted in phosphate-buffered saline to provide conjugated peptides FN-C/H-I, II, III, IV, V, MC-2 and CS1.

Table I

FIBRONECTIN PEPTIDES AND BIOLOGICAL CARRIER MOLECULE

CONJUGATES THEREOF

	<u>(Fibronectin Fragment)</u>	<u>Ovalbumin Conjugate</u>
	FN1 (1906-1924) SEQ ID NO. 1	FN-C/H-I
	YEKPGSPPREVVPRPRPGV	
	FN2 (1946-1960) SEQ ID NO. 2	FN-C/H-II
	KNNQKSEPLIGRKKT	
	FN3 (1721-1960) SEQ ID NO. 3	FN-C/H-III
	YRVRVTPKEKTGPMKE	
	FN4 (1784-1792) SEQ ID NO. 4	FN-C/H-IV
	SPPRRARVT	
	FN5 (1892-1899) SEQ ID NO. 5	FN-C/H-V
	WQPPRARI	
	FN6 (1961-1985) SEQ ID NO. 6	CS-1
	PELPQLVTLPHPNLHGPEILDVPST	
	FN7 (1485-1504) SEQ ID NO. 7	MC-2
	ITVYAVTGRGDSPASSKPISI	

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U.S. Patents 5,019,646; 5,116,368; and 4,839,464 describe peptide fragments from fibronectin which are useful in the present invention.

Human peripheral blood mononuclear cells were isolated from leukophoresis units (NIH Department of Transfusion Medicine) by Ficoll-Hypaque density gradient centrifugation followed by counterflow centrifugal elutriation. The adherent cells were co-cultured with HIV in the presence or absence of anti-CD4 (5 μ g/ml) and the peptides CS-1 and MC-2 (20 μ g/ml). After washing the cells, aliquots of supernatants were harvested at 3-4 day intervals and analyzed for reverse transcriptase activity. Supernatants from the infected cultures were assayed directly for RT activity by a micromethod modified from Spira and co-workers. (J. Geyer and J.S. Apstein, Fifth International Conference on AIDS, International AIDS Society, Montreal, Canada. 4 to 9 June 1989, p. 636. (abstr. Th.C.P. 118). T.J. Spira et al., J. Clin. Microbiol. 25.97 (1987).

As evident in Figure 2, the CS-1 peptide was more effective than the RGD-containing (MC-2) peptide at inhibiting viral infection as monitored by RT activity in the supernatants of cultures infected with HIV. Inhibition was augmented in the presence of anti-CD4. Non-overlapping domains of the 33 kDa fragment of fibronectin also demonstrate suppression of viral infection. Figure 3 compares the reverse transcriptase activity of control cells and cells treated with HIV, C/H-I, C/H-II, C/H-III, C/H-IV and C/H V. Mononuclear leukocytes isolated and cultured as in Figure 2 were infected with HIV in the presence of the indicated peptides at 20 μ g/ml. Supernatants were monitored for reverse transcriptase activity.

In addition, treatment of the mononuclear leukocytes with xyloside which interferes with proteoglycan expression and function, while not independently inhibiting HIV infection of mononuclear leukocytes, acts synergistically with anti-CD4 to block infection, as shown in Figure 4. In this study, mononuclear leukocytes were pre-treated with xyloside (1 ml) for 18 hr and then co-cultured with HIV in the presence or absence of anti-CD4 (5 μ g/ml). Supernatants were monitored for reverse transcriptase activity. These data suggest that binding to monocytes and/or other target cells may involve co-receptor interactions and/or receptor clustering.

Based on these anti-viral activities, the peptides have important therapeutic

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applications. The heparin-binding properties of FN-C/H-I, II and V suggest that they bind to cell surface PG or other molecules on mononuclear leukocytes in addition to integrins, and the identification of this novel heparin-dependent adhesion interaction provides insight into leukocyte infection mechanisms.

5 Polypeptides synthesized can be employed in the present invention in a monovalent state (i.e., free polypeptide or single polypeptide fragment coupled to a biological carrier molecule such as collagen, albumin or other biological or synthetic carrier molecules). Preferably, as described below, conjugates of multiple polypeptide fragments bound to a biological carrier molecule such as ovalbumin
10 (OA), human serum albumin (HSA) and similar proteins. Other biologically acceptable carrier molecules such as polyethylene glycol (PEG) or the like may be utilized. The number of polypeptide fragments associated with or bound to each carrier molecule can be varied, but from about 5 to about 8 polypeptide fragments per carrier molecule are obtained under these coupling conditions. Such modifications
15 can alter the affinity and half-life of the peptide. Antiretroviral agents are generally efficacious in doses ranging from 50 mg/day to about 500 mg/day.

The sterile injectable preparation may be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.
20

25 It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration and rate of excretion.

Example 1

30 General Procedure for Coupling Peptides to Ovalbumin

In a small polypropylene tube add X mg of lyophilized peptide and mg of

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ovalbumin. These proteins are dissolved in water such that the concentration of protein is 2-4 mg of protein per ml of water. In a second polypropylene tube add 10X mg (ten times the peptide amount) of EDC (1-Ethyl-3-[3-Dimethylaminopropyl]-carbodiimide), hydrochloride. The EDC is dissolved in the smallest possible amount of water (to about 25 ul for 20 mg final of EDC) and this solution is added to the tube containing the peptide/ovalbumin mixture. This coupling reaction mixture is mixed in a circular rotator for a minimum of 2 hours at 4°C. The reaction mixture is transferred to dialysis tubing with a 10,000 molecular weight cut-off and dialyzed into 4 liter of Dulbecco's phosphate buffered saline, pH 7.4 (PBS). The dialysis solution is changed every 6 to 8 hours several times to remove the EDC and uncoupled peptides. The volume is adjusted to achieve about 2 mg/ml. of peptide. Aliquots of peptide-conjugate are frozen at -80°C.

Example 2

Treatment of cells and measurement of HIV replication

The fibronectin-derived peptide (20 µl at 2 µg/ml which equals 40 µg/µl final was added during the time of exposure of the mononuclear leukocytes to HIV. The cultures were re-fed at 3 - to 5-day intervals by substituting half of the cell supernatant (500 µl) with fresh medium. Supernatant samples were analyzed (or HIV-1 reverse transcriptase (RT) supernatants from the infected and control adherent mononuclear leukocytes cultures were assayed directly for RT activity by a micromethod modified from Spira and co-workers. J. Geyes and J.S. Epstein, Fifth International Conference on AIDS, International AIDS Society, Montreal, Canada 4 to 9 June 1989, p. 636 (abstr. Th.C.P. 118). T. J. Spira et al., J. Clin. Microbiol. 27, 97 (1987).

The above examples are intended to illustrate the present invention and not to limit it in spirit or scope.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Synthetic Fibronectin Fragments As
Inhibitors of Retroviral Infection

(iii) NUMBER OF SEQUENCES: 7

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(B) COMPUTER: IBM PC compatible
20 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
25 (C) CLASSIFICATION:

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(viii) ATTORNEY/AGENT INFORMATION:

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(C) REFERENCE/DOCKET NUMBER: 92,673

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(C) TELEX: 910-221-5317

(2) INFORMATION FOR SEQ ID NO:1:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: peptide

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(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION: 1..19
(D) OTHER INFORMATION: /note= "FN1 (1906-1924)
5 fibronectin fragment : FN-C/H-I"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Tyr Glu Lys Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg
1906 1910 1915 1920

10 Pro Gly Val
1924

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /note= "FN2 (1946-1960)
15 fibronectin fragment : FN-C/H-II"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys Lys Thr
25 1946 1950 1955 1960

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /note= "FN3 (1721-1960)
35 fibronectin fragment : FN-C/H-III"

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met Lys Glu
 1721 1725 1730 1735

(2) INFORMATION FOR SEQ ID NO:4:

5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..9

(D) OTHER INFORMATION: /note= "FN4 (1784-1792)
fibronectin fragment : FN-C/H-IV"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Pro Pro Arg Arg Ala Arg Val Thr
1784 1788

(2) INFORMATION FOR SEQ ID NO:5:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

(ix) FEATURES:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..8

(D) OTHER INFORMATION: /note= "PN5 (1892-1899)
fibronectin fragment : FN-C/H-V"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Trp Gln Pro Pro Arg Ala Arg Ile
1892 1896

(2) INFORMATION FOR SEQ ID NO:6:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..25

5 (D) OTHER INFORMATION: /note= "FN6 (1961-1985)
fibronectin fragment : CS-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Pro Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu His Gly
1961 1965 1970 1975

10 Pro Glu Ile Leu Asp Val Pro Ser Thr
1980 1985

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids

15 (B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..19

20 (D) OTHER INFORMATION: /note= "FN7 (1485-1504)
fibronectin fragment : MC-2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

25 Ile Thr Val Tyr Ala Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser
1485 1489 1494 1499

Lys Pro Ile Ser Ile
1503

-15-

What is claimed is:

1. A polypeptide comprising a carrier molecule covalently linked to an amino acid sequence selected from the group consisting of FN 1, 2, 3, 4, 5, 6 and 7.
2. A polypeptide according to claim 1 wherein the carrier molecule is ovalbumin.
3. A method for inhibiting retrovirus infection of mammalian cells comprising contacting the cells with an effective inhibiting amount of a fibronectin fragment selected from the group consisting of FN 1, 2, 3, 4, 5, 6 and 7 and carrier molecule conjugates thereof.
4. The method of claim 3 wherein the retrovirus is HIV.
5. The method of claim 3 wherein zidovudine (AZT), dideoxyinosine, dideoxycytosine, or dideoxycytosine is used in combination with the fibronectin fragment alone or conjugated to the carrier molecule.
6. In a method for treating HIV infection with zidovudine (AZT), dideoxyinosine, dideoxycytosine, or dideoxycytosine, the improvement comprising the addition of an effective amount of the polypeptide of claims 1 or 2.

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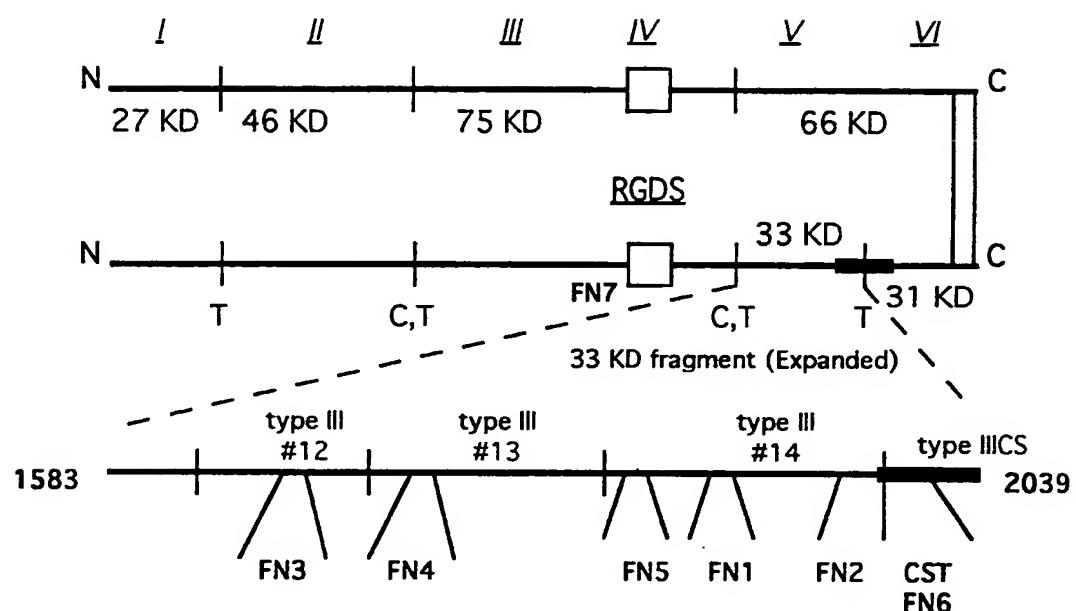


FIGURE 1

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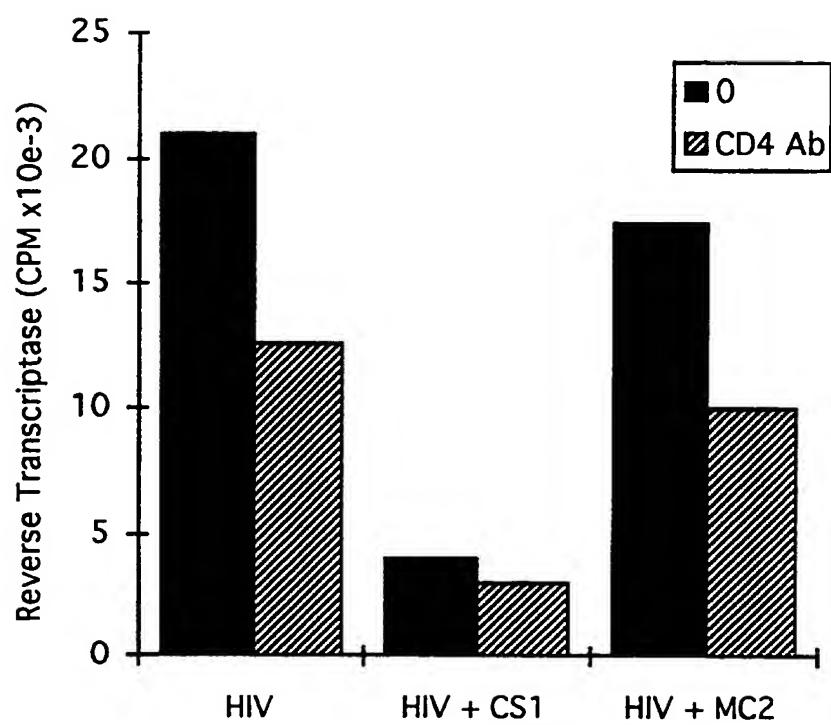


FIGURE 2

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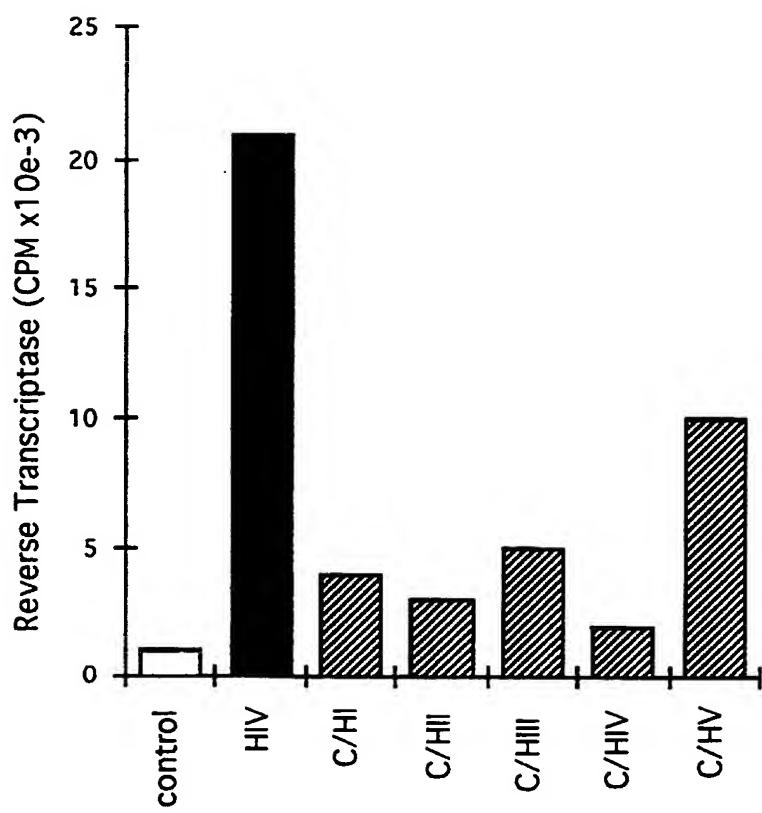


FIGURE 3

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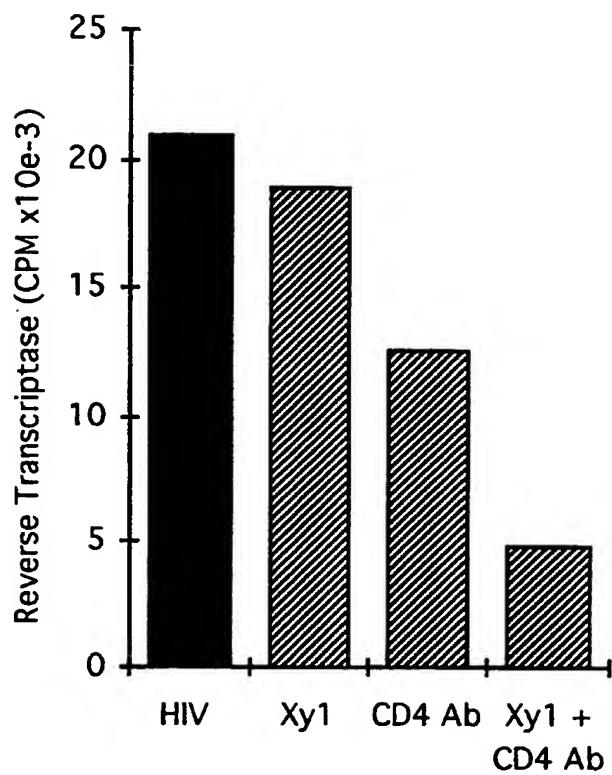


FIGURE 4

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 94/00729

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K7/08 C07K7/06 A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>THE JOURNAL OF NEUROSCIENCE vol. 12, no. 6 , June 1992 pages 2034 - 2042 P.K.HAUGEN ET AL. 'Central and Peripheral Neurite Outgrowth Differs in Preference for Heparin-Binding versus Integrin-Binding Sequences' see page 2025, right column, paragraph 2 -paragraph 3</p> <p>---</p> <p>WO,A,89 01942 (REGENTS OF THE UNIVERSITY OF MINNESOTA) 9 March 1989</p> <p>---</p> <p>-/-</p>	1,2
A		

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Patent family members are listed in annex.

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Date of the actual completion of the international search

28 June 1994

Date of mailing of the international search report

01.07.94

Name and mailing address of the ISA

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THE JOURNAL OF CELL BIOLOGY vol. 114, no. 4 , August 1991 pages 847 - 853 B.S.WEEKS ET AL. 'HIV-1 Infection of Human T Lymphocytes Results in Enhanced alpha5beta1 Integrin Expression' -----	

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 94/00729

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8901942	09-03-89	US-A-	4839464	13-06-89
		US-A-	5019646	28-05-91
		AU-B-	605637	17-01-91
		AU-A-	2385988	31-03-89
		CA-A-	1305084	14-07-92
		DE-A-	3880139	13-05-93
		DE-T-	3880139	21-10-93
		EP-A, B	0366728	09-05-90
		JP-T-	3500046	10-01-91
		US-A-	5116368	26-05-92
		US-A-	5171271	15-12-92
		US-A-	5147797	15-09-92
		US-A-	5294551	15-03-94

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